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THE REGULATION OF CHONDROCYTE DIFFERENTIATION BY RHO GTPASES AND THE ACTIN CYTOSKELETON

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Purpose: There are likely many parallels of genes that regulate chondrocyte differentiation in development and pathology in osteoarthritis. We have identified the role of Rho signaling and the actin cytoskeleton as important regulators of chondrocyte differentiation in development. We have shown that RhoA/ROCK signaling is inhibitory to early stages of chondrocyte differentiation, at the level of suppression of Sox9 transcription. Furthermore, we have identified Rac1 and Cdc42 as promoters of chondrocyte differentiation at the level of regulating the cell adhesion molecule, N-Cadherin and Sox9 transcription, respectively. In parallel to these studies, we have shown that inhibition of actin polymerization by cytochalasin D and promotion of actin polymerization by jasplakinolide promotes chondrogenesis at the level of promoting Sox9 transcription.
Methods: We have extended these studies to the later stages of chondrocyte differentiation to hypertrophy. Using high density monolayer cultures of chondrocytes isolated from embryonic growth plates, we treated cells for 24 hours with the ROCK inhibitor Y27632, cytochalasin D or jasplakinolide, isolated RNA, hybridized to Affymetrix 430 MOE chips and analyzed by microarray. Genes that show at least a two fold change are compared to microarray data obtained in our lab of microdissected embryonic tibia. Common genes are chosen for further studies, by first confirming fold changes by realtime PCR. Normal expression patterns of selected genes are analyzed in embryonic tibia growth plates and compared to growth plates treated with the aforementioned inhibitors.
Results: Our data demonstrate that the majority of genes that are upregulated by cytochalasin D treatment are also upregulated in the hypertrophic region of the growth plate. Functional studies are currently being completed on novel genes that are significantly changed in both hypertrophy and inhibitor treatment. Rorα and FRZB are upregulated in hypertrophy and by cytochalasin D. GDF10 is upregulated by Cytochalasin D, Y27632 treatment and in hypertrophy. Osteomodulin is downregulated by Cytochalasin D, upregulated by Y27632 and hypertrophy. L-plastin is commonly upregulated by all three inhibitors and hypertrophy.
Conclusions: In order to identify targets for osteoarthritis, we need to develop a more conmprehensive understanding of chondrocyte differentiation in development. Rho signaling and it's targets may prove to be quite important in modifying pathologies such as osteoarthritis.

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PERICELLULAR MATRIX MODULATES HEAT SHOCK PROTEIN EXPRESSION BY MECHANICALLY STIMULATED HUMAN CHONDROCYTES

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Purpose: The pathophysiology of osteoarthritis (OA) involves an imbalance between anabolic and catabolic pathways leading to progressive degeneration of the cartilage matrix. In addition to their roles in protein folding, stabilization and transport, heat shock proteins (HSPs) protect cells from cytotoxic effects of stresses such as inflammatory cytokines and nitric oxide. Expression levels of HSPs have been related to OA severity, and gene transfer of HSPs confers cytoprotection in experimental OA. The pericellular matrix (PCM) modulates the mechanical responsiveness of chondrocytes and we have recently reported that retention of the native PCM increases HSP expression in isolated chondrocytes. In this study we investigated the role of the PCM in regulation of HSP expression in response to mechanical stimulation.
Methods: Human articular cartilage was obtained as waste tissue from total joint arthroplasties with approval from the UNC IRB. Chondrocytes and chondrons (chondroyctes with PCM intact) were isolated enzymatically and maintained in alginate bead culture for up to one week before subjecting a subset of each culture from individual specimens to heat shock (1 hr. at 42°C) or cyclic hydrostatic pressure (cHP; 7.5 MPa, 1hz, 4 hours). Matched control samples of each culture were held at 37°C for the duration of treatment. After treatment, cells were recovered from alginate and RNA was isolated and analyzed by real-time rtPCR for expression of HSP 27, HSP 70, HSP 90 and caspase 3 as well as GAPDH for normalization.
Results: There were notable differences between chondrocytes and chondrons in response to both heat shock and cHP. After heat shock expression levels of the three HSPs increased above baseline in both chondrocytes and chondrons ($p<0.01$), but chondrocytes had approximately 2-fold greater activation of HSP 70 and HSP 90 than chondrons (p=0.03, 0.01, resp), while chondrons had 2-fold higher activation of HSP 27 (p=0.02). In response to cHP, HSP 27 did not change significantly in any cultures. HSP 70 was significantly elevated above baseline in chondrocytes (p=0.02) but not chondrons, while HSP 90 expression was elevated in chondrons (p=0.04). There were no significant changes in caspase 3 expression in chondrocytes or chondrons following heat shock or cHP, supporting the conclusion that the stresses did not induce apoptosis.
Conclusions: We have shown that the presence of the native PCM modifies the response of chondrocytes to stress, including mechanical stimulation. We have further demonstrated that primary OA chondrocytes are baro-responsive. Previous reports have suggested that while primary chondrocytes are non-responsive to HP, early changes in OA may cause chondrocytes to lose baro-resistance. HSP 70 and 90, differentially regulated by chondrons and chondrocytes, interact with signaling pathways including caspase 3 and the NF-kB pathway. Our data suggest that the PCM is an important regulator of mechanically induced cell signaling, and alterations to the PCM may affect the progression of OA.

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FUNCTIONAL TESTING OF DIARTHRODIAL JOINT SOFT TISSUES WITH A ROLLING-PLOWING APPARATUS: VALIDATION AND FIRST RESULTS

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Purpose: Dynamic stereometry of the TMJ, i.e. a software reconstruction of real anatomy animated by its real kinematics, combined with numerical modeling can provide in vivo data on strains, forces, stresses, and work density, that improve understanding of craniomandibular biomechanics and are needed for successful biomimetics. Earlier we showed that plowing effects may occur in the TMJ fibrocartilaginous disc, so that this might get damaged being weaker in mediolateral direction. However, the biological response to this complex mechanical environment is still unclear. Therefore, a larger project has been started in order to reproduce the rolling-plowing as recorded in the TMJ (and other diarthrodial joints) on live cartilage explants and analyze the biological reaction to this type of dynamic loading. This presentation will illustrate the construction and validation of